

Table S1. *Cryptococcus gattii* isolates used in this study.

Isolate	Molecular type	Mating type	Year Isolated	Region	Type
EJB3	VGIIa	α	2006	OR, USA	Human
W15209	VGIIa	α	2006	WA, USA	Human
EJB4	VGIIa	α	2007	WA, USA	Human
EJB5	VGIIa	α	2007	WA, USA	Human
EJB6	VGIIa	α	2007	WA, USA	Human
EJB7	VGIIa	α	2007	WA, USA	Human
EJB8	VGIIa	α	2007	WA, USA	Human
EJB9	VGIIa	α	2007	WA, USA	Human
EJB10	VGIIb	α	2007	OR, USA	Human
EJB11	VGIII	α	2008	WA, USA	Human
EJB12	VGIIc	α	2008	OR, USA	Human
EJB13	VGIIa	α	2008	WA, USA	Human
EJB18	VGIIc	α	2008	OR, USA	Human
EJB19	VGIIa	α	2008	OR, USA	Human
3700 (1)	VGIIa	α	2006	WA, USA	Veterinary
3700 (2)	VGIIa	α	2006	WA, USA	Veterinary
3635	VGIIa	α	2006	WA, USA	Veterinary
3059	VGIIa	α	2006	WA, USA	Veterinary
EJB14	VGIIc	α	2007	OR, USA	Veterinary
EJB15	VGIIc	α	2007	OR, USA	Veterinary
EJB16	VGIIa	α	2007	OR, USA	Veterinary
EJB17A+B	VGIIa	α	2008	OR, USA	Veterinary

Table S2. Primers used in this study.

Name	Description	Oligonucleotide Sequence
JOHE10451	<i>SXI1α</i> 5' MLST primer	TACATCACCGGTCATATCTGC
JOHE10452	<i>SXI1α</i> 3' MLST primer	CTGGAGAAGCGCCTCACTGGA
JOHE14115	<i>SXI1α</i> alternative 5' MLST primer	AGGGTACGTTGAGGCCAGTT
JOHE14116	<i>SXI1α</i> alternative 3' MLST primer	GAAAGCGTTGGCAAGGAATGA
JOHE10453	<i>SXI2a</i> 5' MLST primer	TGATCGCACGAGCCAAATCCC
JOHE10454	<i>SXI2a</i> 3' MLST primer	GGCTTCCTGACAACACTTCTA
JOHE14408	<i>IGS</i> 5' MLST primer	ATCCTTGACAGACGACTTGA
JOHE14409	<i>IGS</i> 3' MLST primer	GTGATCAGTGCATTGCATGA
JOHE14976	<i>TEF1</i> 5' MLST primer	GCACGCTCTCGCCTTCAC
JOHE14977	<i>TEF1</i> 3' MLST primer	GTAGTCGGCGTAGGCTCAAC
JOHE14968	<i>GPD1</i> 5' MLST primer	CCACCGAACCTCTAGGATA
JOHE14969	<i>GPD1</i> 3' MLST primer	CTTCTTGGCACCTCCCTGAG
JOHE14970	<i>LAC1</i> 5' MLST primer	AACATGTTCCCTGGGCCTGTG
JOHE14971	<i>LAC1</i> 3' MLST primer	ATGAGAATTGAATGCCCTGT
JOHE14386	<i>CAP10</i> 5' MLST primer	CCGGAACTGACCACTTCATC
JOHE14387	<i>CAP10</i> 3' MLST primer	GCCCCACTCAAGACACAACCT
JOHE14974	<i>PLB1</i> 5' MLST primer	CTCTCATTGTTGCCGCTACT
JOHE14975	<i>PLB1</i> 3' MLST primer	GGAAGCCGAGGTCTGATTG
JOHE14972	<i>MPD1</i> 5' MLST primer	TGCCCTGGATCCTAATGCTCT
JOHE14973	<i>MPD1</i> 3' MLST primer	ACCCAGACTGCCGCTGCGTC
JOHE15459	<i>HOG1</i> 5' MLST primer	AATCTGTGACTTTGGCCTTGC
JOHE15460	<i>HOG1</i> 3' MLST primer	TTCGCTGTACATCATCACCTT
JOHE15431	<i>BWC1</i> 5' MLST primer	CTCCATTCACTGCGCCAATAA
JOHE15432	<i>BWC1</i> 3' MLST primer	ATACGTGCCCTCAAAGATTCT
JOHE15445	<i>CNB1</i> 5' MLST primer	AGTGCCTCATCGTCGATTAGG
JOHE15446	<i>CNB1</i> 3' MLST primer	TCTTCGCTCGAAATCTTCAA
JOHE15471	<i>TOR1</i> 5' MLST primer	TTCCGGTACCATCCTGAGTTAT
JOHE15472	<i>TOR1</i> 3' MLST primer	TTAGCCAAGGTCTTCCCCTG
JOHE15449	<i>CRG1</i> 5' MLST primer	TCAGCACCGCTATTCTTCTTA
JOHE15450	<i>CRG1</i> 3' MLST primer	TACCTAGCACCCGCGTCTCCT
JOHE15451	<i>FHB1</i> 5' MLST primer	TTATGCGTACGCTTGAACAT
JOHE15452	<i>FHB1</i> 3' MLST primer	CCGTCTCCGCTTGACAGAA
JOHE15453	<i>FTR1</i> 5' MLST primer	TCAACTGTACTGACGCTGACC
JOHE15454	<i>FTR1</i> 3' MLST primer	GATGCTCAACTTACACCAACCA
JOHE15437	<i>CAP59</i> 5' MLST primer	TCCGCTGCACAAGTGATACCC
JOHE15438	<i>CAP59</i> 3' MLST primer	CTCTACGTCGAGCAAGTCAAG

Table S3. Previous and novel allele numbers and designations used in this study with GenBank accession numbers.

GenBank Accession number	Submission Details	Reference
AY973641	MLST allele <i>SXI1α_7</i>	Fraser <i>et al</i> 2005
DQ096304	MLST allele <i>SXI1α_12</i>	Fraser <i>et al</i> 2005
DQ096307	MLST allele <i>SXI1α_16</i>	Fraser <i>et al</i> 2005
AY973651	MLST allele <i>SXI1α_17</i>	Fraser <i>et al</i> 2005
DQ096308	MLST allele <i>SXI1α_18</i>	Fraser <i>et al</i> 2005
AY973646	MLST allele <i>SXI1α_19</i>	Fraser <i>et al</i> 2005
AY973647	MLST allele <i>SXI1α_23</i>	Fraser <i>et al</i> 2005
EU937833	MLST allele <i>SXI1α_35</i>	This Study
DQ096311	MLST allele <i>IGS_1</i>	Fraser <i>et al</i> 2005
DQ096313	MLST allele <i>IGS_3</i>	Fraser <i>et al</i> 2005
DQ096314	MLST allele <i>IGS_4</i>	Fraser <i>et al</i> 2005
DQ096318	MLST allele <i>IGS_8</i>	Fraser <i>et al</i> 2005
DQ096319	MLST allele <i>IGS_10</i>	Fraser <i>et al</i> 2005
DQ096323	MLST allele <i>IGS_14</i>	Fraser <i>et al</i> 2005
DQ096324	MLST allele <i>IGS_15</i>	Fraser <i>et al</i> 2005
DQ096327	MLST allele <i>IGS_18</i>	Fraser <i>et al</i> 2005
DQ096358	MLST allele <i>TEF1_1</i>	Fraser <i>et al</i> 2005
DQ096359	MLST allele <i>TEF1_2</i>	Fraser <i>et al</i> 2005
DQ096361	MLST allele <i>TEF1_4</i>	Fraser <i>et al</i> 2005
DQ096362	MLST allele <i>TEF1_5</i>	Fraser <i>et al</i> 2005
DQ096364	MLST allele <i>TEF1_7</i>	Fraser <i>et al</i> 2005
DQ096367	MLST allele <i>TEF1_10</i>	Fraser <i>et al</i> 2005
DQ096377	MLST allele <i>GPD1_1</i>	Fraser <i>et al</i> 2005
DQ096379	MLST allele <i>GPD1_3</i>	Fraser <i>et al</i> 2005
DQ096380	MLST allele <i>GPD1_4</i>	Fraser <i>et al</i> 2005
DQ096381	MLST allele <i>GPD1_5</i>	Fraser <i>et al</i> 2005
DQ096382	MLST allele <i>GPD1_6</i>	Fraser <i>et al</i> 2005
DQ096385	MLST allele <i>GPD1_9</i>	Fraser <i>et al</i> 2005
DQ096392	MLST allele <i>GPD1_16</i>	Fraser <i>et al</i> 2005
DQ096394	MLST allele <i>GPD1_18</i>	Fraser <i>et al</i> 2005
DQ096397	MLST allele <i>LAC_1</i>	Fraser <i>et al</i> 2005
DQ096398	MLST allele <i>LAC_2</i>	Fraser <i>et al</i> 2005
DQ096399	MLST allele <i>LAC_3</i>	Fraser <i>et al</i> 2005
DQ096400	MLST allele <i>LAC_4</i>	Fraser <i>et al</i> 2005
DQ096401	MLST allele <i>LAC_5</i>	Fraser <i>et al</i> 2005
DQ096416	MLST allele <i>CAP10_1</i>	Fraser <i>et al</i> 2005
DQ096417	MLST allele <i>CAP10_2</i>	Fraser <i>et al</i> 2005
DQ096419	MLST allele <i>CAP10_4</i>	Fraser <i>et al</i> 2005
DQ096421	MLST allele <i>CAP10_6</i>	Fraser <i>et al</i> 2005
DQ096422	MLST allele <i>CAP10_7</i>	Fraser <i>et al</i> 2005

DQ096425	MLST allele <i>CAP10_10</i>	Fraser <i>et al</i> 2005
DQ096343	MLST allele <i>PLB1_1</i>	Fraser <i>et al</i> 2005
DQ096344	MLST allele <i>PLB1_2</i>	Fraser <i>et al</i> 2005
DQ096345	MLST allele <i>PLB1_3</i>	Fraser <i>et al</i> 2005
DQ096347	MLST allele <i>PLB1_5</i>	Fraser <i>et al</i> 2005
DQ096348	MLST allele <i>PLB1_6</i>	Fraser <i>et al</i> 2005
DQ096351	MLST allele <i>PLB1_9</i>	Fraser <i>et al</i> 2005
DQ198349	MLST allele <i>PLB1_23</i>	Fraser <i>et al</i> 2005
DQ096330	MLST allele <i>MPD1_1</i>	Fraser <i>et al</i> 2005
DQ096331	MLST allele <i>MPD1_2</i>	Fraser <i>et al</i> 2005
DQ096332	MLST allele <i>MPD1_3</i>	Fraser <i>et al</i> 2005
DQ096334	MLST allele <i>MPD1_5</i>	Fraser <i>et al</i> 2005
DQ096337	MLST allele <i>MPD1_8</i>	Fraser <i>et al</i> 2005
DQ096456	MLST allele <i>HOG1_1</i>	Fraser <i>et al</i> 2005
DQ096457	MLST allele <i>HOG1_2</i>	Fraser <i>et al</i> 2005
EU937830	MLST allele <i>HOG1_3</i>	This Study
EU937831	MLST allele <i>HOG1_4</i>	This Study
EU937832	MLST allele <i>HOG1_5</i>	This Study
DQ096428	MLST allele <i>BWC1_1</i>	Fraser <i>et al</i> 2005
EU937818	MLST allele <i>BWC1_2</i>	This Study
DQ096442	MLST allele <i>CNB1_1</i>	Fraser <i>et al</i> 2005
DQ096443	MLST allele <i>CNB1_2</i>	Fraser <i>et al</i> 2005
EU937820	MLST allele <i>CNB1_3</i>	This Study
DQ096470	MLST allele <i>TOR1_1</i>	Fraser <i>et al</i> 2005
DQ096471	MLST allele <i>TOR1_2</i>	Fraser <i>et al</i> 2005
DQ096444	MLST allele <i>CRG1_1</i>	Fraser <i>et al</i> 2005
DQ096445	MLST allele <i>CRG1_2</i>	Fraser <i>et al</i> 2005
EU937821	MLST allele <i>CRG1_3</i>	This Study
EU937822	MLST allele <i>CRG1_4</i>	This Study
EU937823	MLST allele <i>CRG1_5</i>	This Study
DQ096446	MLST allele <i>FHB1_1</i>	Fraser <i>et al</i> 2005
DQ096447	MLST allele <i>FHB1_2</i>	Fraser <i>et al</i> 2005
EU937824	MLST allele <i>FHB1_3</i>	This Study
EU937825	MLST allele <i>FHB1_4</i>	This Study
EU937826	MLST allele <i>FHB1_5</i>	This Study
DQ096448	MLST allele <i>FTR1_1</i>	Fraser <i>et al</i> 2005
DQ096449	MLST allele <i>FTR1_2</i>	Fraser <i>et al</i> 2005
EU937827	MLST allele <i>FTR1_4</i>	This Study
EU937828	MLST allele <i>FTR1_5</i>	This Study
EU937829	MLST allele <i>FTR1_6</i>	This Study
DQ096432	MLST allele <i>CAP59_1</i>	Fraser <i>et al</i> 2005
DQ096433	MLST allele <i>CAP59_2</i>	Fraser <i>et al</i> 2005
DQ096434	MLST allele <i>CAP59_3</i>	Fraser <i>et al</i> 2005
EU937819	MLST allele <i>CAP59_4</i>	This Study

Supplementary Methods

Case and Isolate Identification. Melanin production was assayed by growth and dark pigmentation on Staib medium, and urease activity was detected by growth and alkaline pH change on Christensen's Agar. These tests establish that isolates were either *C. neoformans* or *C. gattii*. Isolates were then examined for resistance to canavanine and utilization of glycine on L-canavanine, glycine, 2 bromothymol blue (CGB) agar. Growth on CGB agar indicates that isolates are canavanine resistant, and able to use glycine as a sole carbon source, triggering a bromothymol blue color reaction indicative of *C. gattii*, whereas *C. neoformans* is sensitive to canavanine, and cannot use glycine as a sole carbon source, resulting in no growth or coloration in this selective indicator medium. All positive samples were colony purified and grown on rich medium prior to extraction of genomic DNA.

Genotyping. All primers used for MLST analysis were designed specifically to amplify ORF gene sequence regions including variable non-coding DNA regions to maximize discriminatory power [3] (Table S2). Sequences from both forward and reverse strands were assembled, and manually edited using Sequencher version 4.8 (Gene Codes Corporations). Using BLAST analysis of the GenBank database (NCBI), each allele was assigned a number based on Fraser *et al.* 2005, or assigned a new allele number if a perfect match was not found (Table S3). GenBank accession numbers with corresponding allele numbers are listed in the supplementary information (Table S3).